DISAL glycosyl donors for efficient glycosylations under acidic conditions: Application to solid-phase oligosaccharide synthesis [1,2]

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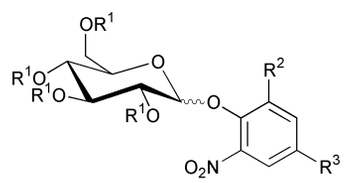
The use of DISAL (methyl *di*nitro*sal*icylate) glycosyl donors in efficient Lewis acid-promoted glycosylations is reported. *N*-Acetyl-D-glucosamine monosaccharide acceptors are successfully glycosylated at O-6 or O-4 using benzyl- and benzoyl-protected DISAL donors in CH_2Cl_2 or nitromethane in the presence of $LiClO_4$. The resultant disaccharides are isolated in yields ranging from 35 to 93%. Other Lewis acids such as $FeCl_3$, TMSOTf, or $BF_3{\cdot}Et_2O$ also prove efficient for glycosylation of the secondary alcohol cyclohexanol. However, for the synthesis of disaccharides, the mild activation by $LiClO_4$ gives higher yields. This approach is extended to efficient solid-phase glycosylation of a D-glucosamine derivative anchored by the 2-amino group through a Backbone Amide Linker (BAL) to a polystyrene support.

Introduction

Cell-surface oligosaccharides are involved in many biological recognition processes including leukocyte–endothelial cell adhesion (leukocyte recruitment in inflamed tissue), bacterial and viral infection, and immunological recognition of tumor cells and foreign tissue in xenotransplantation.[3] Also, free oligosaccharides can have biological effects such as the rhizobial lipochitin oligosaccharides, which function as nodulation factors.[4] Amino sugars are found in many biologically important poly- and oligosaccharides, *e.g.* in glycans of *O*- and *N*-glycoproteins and peptides, lipochitin nodulation factors, and amino glycoside antibiotics such as streptomycin.

Established methods for glycosylation of aliphatic alcohols, most notably glycosyl bromides, fluorides, trichloroacetimidates, sulfoxides, and *n*-pentenyls as well as thioglycosides and glycals, all require activation by a Lewis acid [*e.g.*, AgOTf, $BF_3{\cdot}Et_2O$, TMSOTf, or dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST)] prior to the actual glycosylation reaction. Waldmann and co-workers have reported activation of glycosyl fluorides, trichloroacetimidates, and phosphites using the mild Lewis acid $LiClO_4$.[5] This has been ascribed to the ability of solutions of $LiClO_4$ in Et_2O to stabilise polar or ionic transition states.[5] Lubineau and Drouillat have reported LiOTf as an alternative to $LiClO_4$ as a promoter in glycosylation reactions and ascribed its effect to general acid catalysis.[6] In a previous paper we have described an efficient method for glycosylation under strictly neutral or mild basic conditions.[7] In this glycosylation technique, the anomeric leaving group on benzyl-protected donors is methyl 3,5-*di*nitro*sal*icylate[8] (DISAL) **1** or its *para* regioisomer **2** (Fig. 1).

These aryl glycosides were prepared by convenient nucleophilic aromatic substitution of an activated aryl fluoride and were stable on prolonged storage at 5 °C and for days in CH_2Cl_2 and other non-polar solvents. They become efficient glycosyl donors in polar, aprotic solvents, such as *N*-methylpyrrolidin-2-one (NMP), in the *absence* of Lewis acids. Methanol was glycosylated *stereospecifically* and less reactive alcohols, *i.e.* monosaccharides, were glycosylated with α-selectivity. This concept has also been extended to intramolecular glycosylations.[9] Whereas *benzyl*-protected aryl glycosides were efficient glycosyl donors under neutral conditions, the analogous *benzoyl*-protected donors did not give the expected glycosides, in part due to trapping of intermediates as the orthoesters.



1α,β R^1 = Bn, R^2 = CO_2Me, R^3 = NO_2
2α,β R^1 = Bn, R^2 = NO_2, R^3 = CO_2Me
3α,β R^1 = Bz, R^2 = CO_2Me, R^3 = NO_2

Fig. 1 Generalised structure of DISAL glycosyl donors.

Solid-phase synthesis has been tremendously successful for the synthesis of peptides and oligonucleotides, and even small proteins, due to its inherent simplicity and automatability.[10] Interest in the solid-phase synthesis of small organic molecules has escalated over the past decade.[11] When solid-phase syntheses are carried out combinatorially, large numbers of structurally diverse compounds can be accessed.[12] The most notable failure of solid-phase synthesis of biopolymers is in the field of oligosaccharide synthesis. Despite numerous efforts, there is still no truly *general* strategy for the solid-phase synthesis of oligosaccharides.[13–15]

Here we report on the use of both benzyl- and benzoyl-protected DISAL glycosyl donors in the presence of 'classical' Lewis acid glycosylation promoters, such as $BF_3{\cdot}Et_2O$, TMSOTf, and $FeCl_3$.[16] Furthermore, the use of $LiClO_4$ as a mild activator is reported as an efficient and preferred alternative to these Lewis acids. Finally, DISAL glycosyl donors were applied to solid-phase oligosaccharide synthesis using a Backbone Amide Linker (BAL) strategy for anchorage of D-glucosamine derivatives.

Results and discussion

Model glycosylation of cyclohexanol

Initial studies focused on glycosylation of the secondary alcohol cyclohexanol as a model for carbohydrate acceptors.

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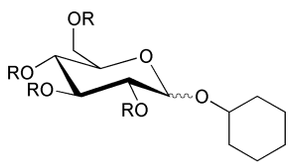
Table 1 Model glycosylation of cyclohexanol using benzyl-protected donor **1**

Entry	Donor[a]	Activator (equiv.)	Solvent	Time (*t*/h)	Temp. (*θ*/°C)	Yield of **4** (%) (α/β)[b]
1	**1**	$BF_3{\cdot}Et_2O$ (2.0)	Toluene	17	rt	97 (2.1 : 1)
2	**1**	TMSOTf (1.1)	Toluene	17	rt	87 (2.2 : 1)
3	**1**	$FeCl_3$ (2.0)	CH_2Cl_2	2	rt	89 (1.5 : 1)
4	**1**	$LiClO_4$ (2.5)	CH_2Cl_2	17	rt	93 (1 : 1.1)
5	**1**	$LiClO_4$ (2.5)	Et_2O	17	rt	84 (2.2 : 1)
6	**1**	$LiClO_4$–Bu_4NI (2.5)	CH_2Cl_2	17	rt	81 (6.4 : 1)
7	**1**	Bu_4NI (2.0)	CH_3NO_2	17	50	90 (4.3 : 1)

[a] α/β 4 : 1. [b] Determined by RP-HPLC (215 nm).



4α,β R = Bn
5α,β R = Bz

Fig. 2 Structure of model cyclohexyl glycoside products.

Cyclohexyl glycosides produced (Fig. 2) were readily separated by analytical RP-HPLC, thus providing information on the reaction yield and α/β ratio. To assess the efficiency of the donor, cyclohexanol was used in excess (5 equiv.). Early experiments pointed to either CH_2Cl_2 or toluene as reaction medium. $(CH_2Cl)_2$ was used as a higher-boiling equivalent of CH_2Cl_2, and CH_3CN or CH_3NO_2 as polar solvents.

Treatment of benzyl-protected donor **1** with cyclohexanol at room temperature in the presence of $BF_3{\cdot}Et_2O$ revealed that at least stoichiometric amounts of Lewis acid were required for the glycosylation to proceed. Thus, for the reaction in toluene, sub-stoichiometric amounts of $BF_3{\cdot}Et_2O$ left much of the donor unchanged after 17 h, 1.1 equiv. left only a trace of donor, and 2 equiv. produced cyclohexyl glycoside **4** in 97% yield (Table 1, Entry 1). Interestingly, the α-anomer was consumed first in this reaction. TMSOTf proved to be more activating, as only a trace of donor was observed with 0.5 equiv. after 17 h and total conversion was achieved with 1.1 equiv. to yield 87% of **4** (Entry 2). $FeCl_3$, solubilised in CH_2Cl_2, also proved efficient and 2 equiv. gave total conversion to 89% of **4** after only 2 h (Entry 3).

The requirement for more than stoichiometric amounts of $BF_3{\cdot}Et_2O$ deserves a comment. It has been reported that 4-methoxysalicylaldehyde readily forms a tight complex with BF_3, releasing one equivalent of HF.[17] We speculated that the released phenoxide anion of methyl 2-hydroxy-3,5-dinitrobenzoate was not only acting as a *general* Lewis base towards Lewis acids, but also reacted with BF_3 in a *specific* manner similar to its reaction with 4-methoxysalicylaldehyde.

It was rewarding to see that the very mild Lewis acid $LiClO_4$ also proved efficient in activating donor **1**. Even though only sparingly soluble in CH_2Cl_2, $LiClO_4$ readily gave a near quantitative yield of **4** as an α/β mixture (Entry 4). The effect of the solvent on anomeric selectivity was studied. Incomplete reaction was observed in CH_3CN (data not shown). However, full conversion and slightly higher α-selectivity was observed in Et_2O (Entry 5). Addition of an auxiliary nucleophile, Bu_4NI, to the glycosylation in CH_2Cl_2 in the presence of $LiClO_4$ gave both a good yield and high α-selectivity (Entry 6). An NMR experiment with donor **1**, Bu_4NI, and $LiClO_4$ (2 equiv. each) in $CDCl_3$ at room temperature showed a doublet at δ 6.84 (J 3.8 Hz)[18] characteristic for the α-glucosyl iodide after only 30 min (≈ 20% conversion). With Bu_4NI alone, good yield and α-selectivity was achieved but it required slightly more forcing conditions (Entry 7).

Next, we turned to glycosylation of cyclohexanol with *benzoyl*-protected donor **3** which had proven inefficient under neutral conditions. Using up to 10 equiv. of $BF_3{\cdot}Et_2O$ or TMSOTf at room temperature left most of the donor unchanged after 17 h (data not shown). However, raising the temperature to 40 °C in the presence of TMSOTf in 1,2-dichloroethane, toluene or CH_3NO_2 resulted in complete consumption of the donor and formation of **5** in 82, 81, and 71% yield, respectively (Table 2, entries 1–3). The two anomers of donor **3** had significantly different reactivities in the glycosylation of cyclohexanol in the presence of $BF_3{\cdot}Et_2O$. Whereas **3α** gave incomplete conversion (data not shown), donor **3β** gave 86% of **5** after 17 h with complete consumption of the donor (Entry 4). In the case of $FeCl_3$, in the presence of 4 Å molecular sieves, no reaction occurred for **3α** whereas **3β** gave the pure β-product in 67% yield (Entries 5 and 6). Again, $LiClO_4$ proved mild and efficient in activating **3β** in CH_3NO_2. After 17 h at 40 °C, **5β** was obtained in 81% yield and no **5α** could be detected (Entry 7).

In some of the above experiments, the kinetically favored β-glycoside, which was formed initially, under longer reaction times converted into the thermodynamically favored α-glycoside, most likely due to strong Lewis acid-promoted transglycosylation in the presence of excess of acceptor, cyclohexanol (Entries 1–4).

Thus, in the initial screening of Lewis acid-promoted glycosylations with DISAL donors, $BF_3{\cdot}Et_2O$ and $LiClO_4$ proved to be the most efficient promoters for glycosylation of cyclohexanol with donors **1** and **3**. Best results for **3** were provided by the β-donor, as the α-anomer was less reactive. Consequently, **3β** was used in the following glycosylations, in addition to **1**.

Glycosylation of carbohydrate acceptors

With these optimised conditions in hand, we turned to glycosylation of monosaccharide acceptors, first with benzyl-protected donor **1α,β**. We initially used diisopropylidene-protected acceptors **6** and **7** (Scheme 1), which could be glycosylated by DISAL donors under neutral conditions.[7] However, they proved not to be sufficiently stable in the presence of $BF_3{\cdot}Et_2O$. Turning to the more stable *benzyl*-protected primary and secondary acceptors **8**[19] and **9**,[20] respectively, moderate yields of the 1,6- and 1,4-linked disaccharides **10** and **11**, respectively, were obtained (Table 3, Entries 1 and 2). However, lowering the temperature to 0 °C and shortening the reaction time gave rise to a significantly improved yield of 82% of **10** (Entry 3). Lowering the temperature further to −44 °C slowed the reaction significantly and at −78 °C no glycosylation was observed (data not shown). Also, substituting $BF_3{\cdot}Et_2O$ by either $FeCl_3$ or TMSOTf in the glycosylation with **1** in CH_2Cl_2 gave rise to complex reaction mixtures (data not shown).

The apparent breakdown of initially formed disaccharide in the presence of $BF_3{\cdot}Et_2O$ in extended reactions at rt is noteworthy, as glycosides generally are considered stable to $BF_3{\cdot}Et_2O$. We assumed that the HF generated by formation of the above-mentioned complex between $BF_3{\cdot}Et_2O$ and released

Table 2 Model glycosylation of cyclohexanol using benzoyl-protected donor **3**

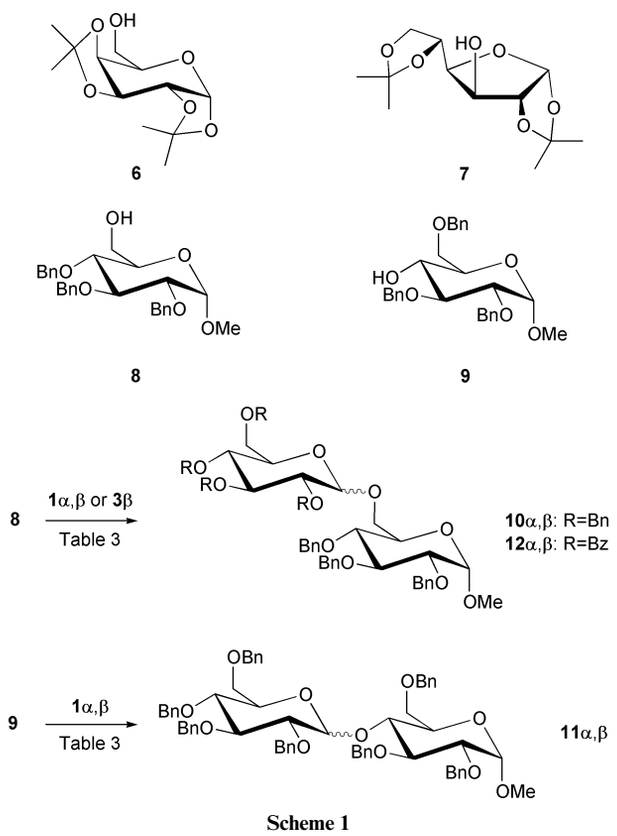
Entry	Donor	Activator (equiv.)	Solvent	Time (*t*/h)	Temp. (*θ*/°C)	Yield of **5** (%) (α/β)[a]
1	**3**	TMSOTf (10)	$(CH_2Cl)_2$	17	40	82 (7.2 : 1)
2	**3**	TMSOTf (10)	Toluene	17	40	81 (6.7 : 1)
3	**3**	TMSOTf (10)	CH_3NO_2	17	40	71 (7.2 : 1)
4	**3β**	$BF_3{\cdot}Et_2O$ (5.0)	$(CH_2Cl)_2$	17	40	86 (9.6 : 1)
5	**3α**	$FeCl_3$ (5.0)[b]	CH_3CN	17	40	[c]
6	**3β**	$FeCl_3$ (5.0)[b]	CH_3CN	17	40	67 (β only)
7	**3β**	$LiClO_4$ (2.5)	CH_3NO_2	17	40	81 (β only)

[a] Determined by RP-HPLC (215 nm). [b] 4 Å molecular sieves. [c] No reaction.

Table 3 Synthesis of disaccharides

Entry	Donor[a]	Acceptor	Activator (equiv.)	Solvent	Time (*t*/h)	Temp. (*θ*/°C)	Product	Yield (%) (α/β)[b]
1	**1α,β**	**8**	$BF_3{\cdot}Et_2O$ (1.8)	CH_2Cl_2	17	rt	**10**	40 (3.3 : 1)
2	**1α,β**	**9**	$BF_3{\cdot}Et_2O$ (1.8)	CH_2Cl_2	17	rt	**11**	34 (1 : 3.3)
3	**1α,β**	**8**	$BF_3{\cdot}Et_2O$ (1.8)	CH_2Cl_2	0.5	0	**10**	82 (1 : 1)
4	**3β**	**8**	$BF_3{\cdot}Et_2O$ (1.8)[c]	toluene	17	40	**12**	46 (β)
5	**1α,β**	**14**	$BF_3{\cdot}Et_2O$ (1.8)[c]	CH_2Cl_2	17	rt	**16**	77 (1.4 : 1)
6	**1α,β**	**14**	$LiClO_4$ (2.0)[c,d]	CH_2Cl_2	1.5	rt	**16**	93 (1.6 : 1)
7	**3β**[e]	**14**	$LiClO_4$ (3.7)	CH_3NO_2	17	40	**17**	91 (β)
8	**1α,β**	**19**	$LiClO_4$ (2.0)[c,d]	CH_2Cl_2	5	rt	**22**	82 (1.9 : 1)
9	**3β**	**19**	$LiClO_4$ (3.7)	CH_3NO_2	17	40	**23**	35 (β)

[a] 1.5 equiv. donor. [b] Isolated yield. [c] Molecular sieves 4 Å. [d] 2.0 equiv. of Li_2CO_3 added. [e] 2.0 equiv. donor.



Scheme 1

phenoxide caused the decomposition of formed products. Several scavengers were tested to quench formed HF, *e.g.* silicon-containing compounds and sterically hindered bases (data not shown). We found that addition of molecular sieves suppressed the decomposition significantly and allowed for glycosylation at higher temperatures and longer reaction times. Thus, primary acceptor **8** was successfully glycosylated by benzoyl-protected donor **3β** at 40 °C in toluene in the presence of $BF_3{\cdot}Et_2O$ to give β-glycoside **12β** in 46% yield (Entry 4).

Next, we turned to the glycosylation of D-glucosamine derivatives. Intermediate **13** (Scheme 2) was synthesized starting from *N*-acetyl-D-glucosamine by Fischer glycosylation, followed by tritylation[21] of the 6-OH, and benzylation.[22] This was achieved in good yields with no chromatographic steps. Selective hydrolysis of the trityl ether gave primary acceptor **14**. Furthermore, hydrolysis of **13** under more forcing conditions gave amine **15** in good yield. Glycosylation of **14** in CH_2Cl_2 at room temperature with benzyl donor **1** activated by $BF_3{\cdot}Et_2O$ yielded 77% of disaccharide **16α,β** (Scheme 2; Table 3, Entry 5). A significantly improved yield was obtained by activation with $LiClO_4$ and product **16**α,β was isolated in 93% yield, again as an anomeric mixture (Entry 6). Note at this point that addition of solid Li_2CO_3 as an acid scavenger was tolerated by the protocol. This quenched or slowed down the activation when using $BF_3{\cdot}Et_2O$. Acceptor **14** was also glycosylated successfully with donor **3β** and the pure β-product **17β** was obtained in an excellent 91% yield (Entry 7).

To obtain the corresponding acceptor with a free O-4, the known benzylidene **18** was selectively ring-opened[20] using triethylsilane and $BF_3{\cdot}Et_2O$, and the desired product **19** was isolated in 74% yield. Furthermore, benzylidene **18** was selectively *N*-deacetylated in good yield (84%) with Tf_2O and pyridine following the procedure by Charette and Chua.[23] This procedure was originally developed for the conversion of mono- and disubstituted amides into esters and free amines, but was readily adapted for *N*-deacetylation of GlcNAc derivatives. Resultant benzylidene **20** was subsequently ring-opened by prolonged treatment with triethylsilane and $BF_3{\cdot}Et_2O$ and a moderate yield (55%) of amine **21** was isolated. The 1⟶4 linkage to GlcNAc acceptors is among the most difficult to establish. It was rewarding to see that secondary acceptor **19** was glycosylated efficiently using an excess of $LiClO_4$ in CH_3NO_2. Thus, using benzyl donor **1α,β**, disaccharide **22**α,β was isolated as an anomeric mixture in 82% yield (Entry 8). For the benzoyl donor, disaccharide **23** was obtained as the pure β-anomer in a moderate isolated yield (Entry 9).

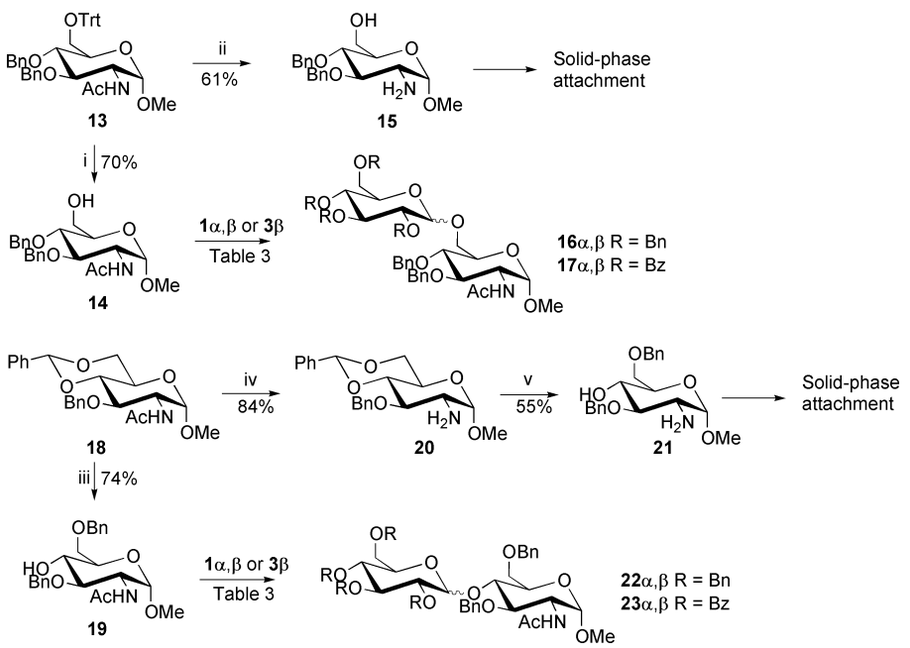
Solid-phase glycosylations

For solid-phase glycosylations, we relied on a BAL strategy[24] for anchoring of D-glucosamine derivatives. This allows for safety-catch attachment through the amine, thereby providing high stability towards Lewis acids during glycosylation and

Table 4 Solid-phase glycosylation of polystyrene-bound acceptors **25** and **26**

Entry	Donor (equiv.)	Resin	Activator (equiv.)	Solvent	Temp. (θ/°C)	Product	Yield (%)[a]	Recovery (%)[b]
1	**1** (3.0)	**25**	$BF_3{\cdot}Et_2O$ (3.6)	CH_2Cl_2	rt	**16**	12	97
2	**1** (5.0)	**25**	$BF_3{\cdot}Et_2O$ (6.0)	CH_2Cl_2	rt	**16**	25	86
3	**1** (10.0)	**25**	$BF_3{\cdot}Et_2O$ (12.0)	CH_2Cl_2	rt	**16**	80	79
4	**3** (3.0)	**25**	$BF_3{\cdot}Et_2O$ (3.6)	Toluene	40	**17**	1	98
5	**3** (5.0)	**25**	$BF_3{\cdot}Et_2O$ (6.0)	Toluene	40	**17**	17	90
6	**3** (10.0)	**25**	$BF_3{\cdot}Et_2O$ (12.0)	Toluene	40	**17**	75	83
7	**1** (10.0)	**25**	$LiClO_4$ (25)	CH_2Cl_2	rt	**16**	98	65
8	**1** (10.0)	**26**	$LiClO_4$ (25)	CH_2Cl_2	rt	**22**	52	79
9	**3** (15.0)	**25**	$LiClO_4$ (38)	CH_3NO_2	40	**17**	88	83
10	**3** (15.0)	**26**	$LiClO_4$ (38)	CH_3NO_2	40	**23**	7	97
11	**1** (10.0)	**25**	Bu_4NI (30)	CH_3NO_2	50	**16**	84	22

[a] Ratio of product to the sum of unchanged acceptor and product calculated from standard curves on RP-HPLC (215 nm). [b] Sum of unchanged acceptor (**27** or **28**) and disaccharide (**16**, **17**, **22**, or **23**) compared with initially measured loading.

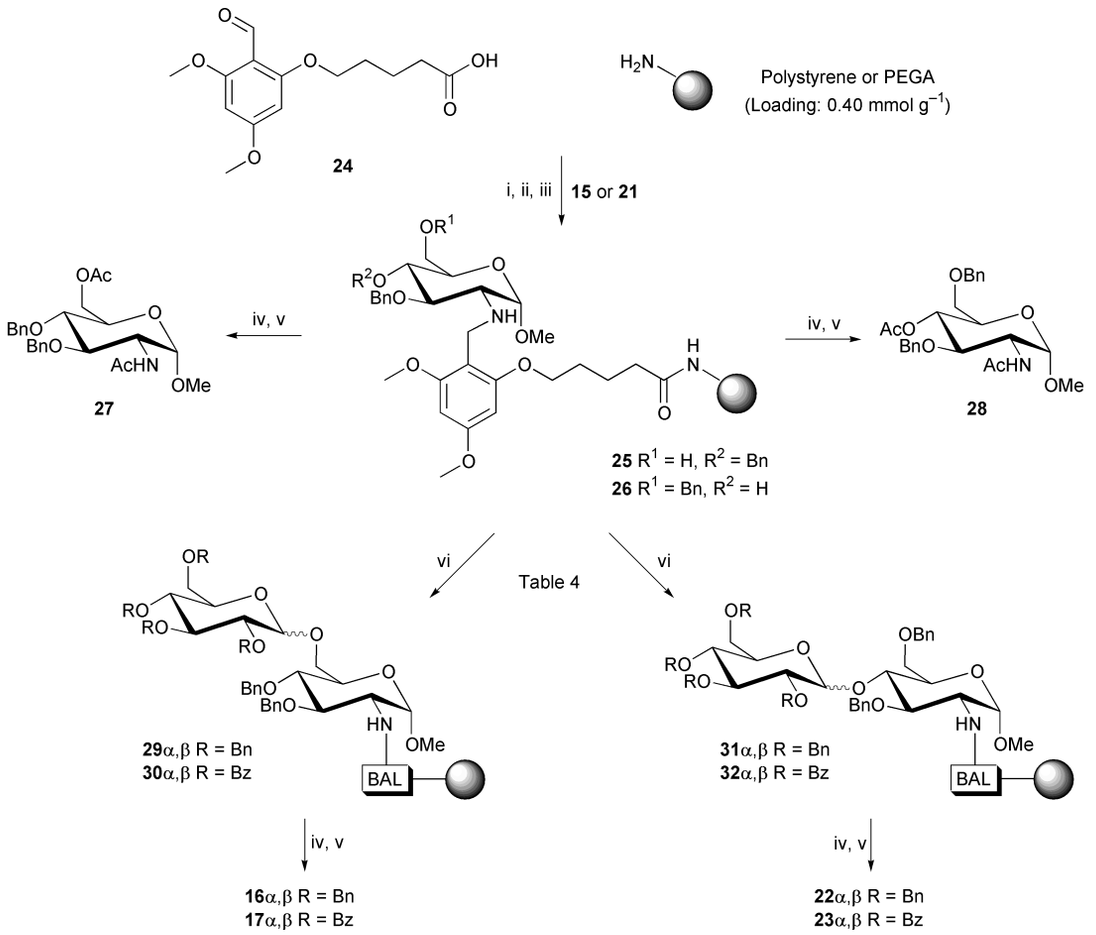


Scheme 2 *Reagents and conditions*: (i) THF–3 M aq. HCl (5 : 1), 50 °C, 17 h; (ii) THF–3 M aq. HCl (2 : 1), reflux, 3 d; (iii) Et_3SiH, $BF_3{\cdot}Et_2O$ (2 equiv.), CH_2Cl_2, 0 °C; (iv) Tf_2O, pyridine, −40 °C; then excess of EtOH; (v) Et_3SiH, $BF_3{\cdot}Et_2O$ (3 equiv.), CH_2Cl_2, 0 °C.

efficient release of final products by TFA after acetylation of the nitrogen.

First, 5-(2-formyl-3,5-dimethoxyphenoxy)valeric acid (*o*-PALdehyde, **24**) was coupled to aminomethylated polystyrene (PS) or poly(ethylene glycol) polyacrylamide (PEGA) supports, both with an initial loading of 0.40 mmol g^{-1} using a standard peptide protocol.[14] Next, primary acceptor **15** was anchored by reductive amination in the presence of $NaBH_3CN$. Reductive amination in DMF–AcOH (99 : 1)[25] proceeded well on a PS support but gave low yields on PEGA.[26] Previously, it was observed that reductive amination on PALdehyde *in solution* gave double alkylation in DMF but monoalkylation in methanol.[24a] We reasoned that the apparent low loading was due to double alkylation of the amino group and attributed this to reaction with two PALdehyde moieties in flexible PEGA supports due to lack of efficient 'site isolation'.[26] Thus, when the reductive amination on PEGA was carried out in methanol, good yields were obtained. After acetylation, monosaccharide product **27** was released with TFA–water (19 : 1) (Scheme 3), which gave a loading of 0.30 mmol g^{-1} for PS (94%, 5 steps) and 0.028 mmol g^{-1} for PEGA (80%, 5 steps); the latter swelled in NMP. PEGA proved difficult to handle in the dried state, and therefore was handled swelled in dry NMP. Loading was calculated from drained weight, *i.e.* swelled in NMP, thus giving a seemingly low loading. Reductive amination with amine **21** on PS-supported *o*-PALdehyde **24** followed by release of acetylated, secondary acceptor **28** gave a loading of 0.27 mmol g^{-1} (84%, 5 steps). Acetylated acceptors **27** and **28** were both identified by NMR and MS. It should be noted that addition of DMAP to the acetylation mixtures should be avoided as it gave rise to impurities.

Initial efforts on solid-phase glycosylation with DISAL donors started from the previously reported *neutral* conditions in solution.[7] However, the benzyl-protected donor **1α,β** (up to 10 equiv.) in NMP at 40–80 °C glycosylated PS- and PEGA-bound acceptor in only 0–9% yield. Next, we used Lewis acid-promoted glycosylations as above. Promoters TMSOTf and $FeCl_3$ proved unsuitable for glycosylations with donors **1α,β** or **3β** on the solid phase. However, with donor **1α,β** in the presence of $BF_3{\cdot}Et_2O$ and 4 Å molecular sieves, efficient glycosylation of polystyrene-bound acceptor **25** was achieved. Under the same conditions, the PEGA-bound acceptor was not glycosylated, presumably due to quenching of the Lewis acid by the resin. Glycosylations on PS, on the other hand, gave disaccharide **16α,β** (α/β ≈ 2 : 1) and monosaccharide **27** in ratios of 12 : 88, 25 : 75 and 80 : 20 for a glycosylation using 3, 5, or 10 equiv. of **1α,β**, respectively (Table 4, Entries 1–3). Substituting glycosyl donor **1α,β** by the more reactive **2α,β** did not increase the yield (data not shown). Benzoyl-protected donor **3β** in toluene at 40 °C in the presence of $BF_3{\cdot}Et_2O$ yielded ratios of the expected disaccharide **17β** to monosaccharide **27** of 1 : 99, 17 : 83 and 75 : 25, again with 3, 5, or 10 equiv. of donor, respectively



Scheme 3 *Reagents and conditions*: (i) HOBt (2 equiv.), HBTU (1.9 equiv.), DIEA (3.9 equiv.), DMF, rt, 16 h; (ii) CH_2Cl_2–Ac_2O–pyridine (18 : 1 : 1), 16 h; (iii) **15** or **21** (2 equiv.), $NaBH_3CN$ (10 equiv.), DMF–AcOH (99 : 1) (PS) or MeOH–AcOH (99 : 1) (PEGA); (iv) Ac_2O–pyridine (2 : 1), 16 h; (v) TFA–water (19 : 1), 60 min; (vi) glycosylation: see Table 4.

(Entries 4–6). Thus, **3β** also proved to be efficient in glycosylation reactions on the solid phase.

We then turned to $LiClO_4$ activation of glycosyl donors in solid-phase glycosylations. Donor **1** (10 equiv.) again proved efficient and gave an almost quantitative conversion to disaccharide **16** (Entry 7), however, with a lower recovery than for $BF_3{\cdot}Et_2O$ (Entry 3). The very hindered resin-bound acceptor **26** was also glycosylated under these conditions to give a 52% yield of disaccharide **22** (Entry 8). Donor **3β** with activation by $LiClO_4$ also glycosylated both resin-bound acceptors **25** and **26** to give disaccharides **17** and **23** in 88% (α/β 10 : 1; Entry 9) and 7% yield, respectively (Entry 10). The conditions under which cyclohexanol was efficiently glycosylated using donor **1** together with Bu_4NI (Table 1; Entry 7) were also tested in solid-phase glycosylation of **25**. A high conversion was achieved together with a high α-selectivity (α/β 13 : 1, Entry 11). However, a significant amount of material appeared to be lost from the resin under these conditions.

Thus, a high degree of glycosylation of O-6 of a resin-bound glucosamine acceptor was achieved using 10–15 equiv. of donors in the presence of a large excess of the very mild Lewis acid $LiClO_4$ in solutions of CH_3NO_2 or CH_2Cl_2 as solvent.

Conclusions

We have described the activation of DISAL glycosyl donors by Lewis acids to promote glycosylation reactions in *non-polar* solvents. Whereas *benzoyl*-protected DISAL donors previously had proven inefficient for glycosylation under neutral conditions, Lewis acids activated these 'disarmed' donors efficiently. While some conventional promoters of glycosylations, such as $BF_3{\cdot}Et_2O$ and TMSOTf, also proved their efficiency here, it was remarkable that the very mild Lewis acid $LiClO_4$ also was a competent promoter. *Benzyl*-protected DISAL donors gave predominantly the α-glycosides, whereas the *benzoyl*-protected donor in general gave β-glycosides. The preferred conditions for glycosylations at O-6 and O-4 were 1.5 equiv. of donor in a solution of $LiClO_4$ at rt (CH_2Cl_2) or 40 °C (CH_3NO_2). Using this protocol, disaccharides were prepared in up to 93 and 82% yield for the (1⟶6)- and (1⟶4)-linkage, respectively. Finally, the optimised conditions were adapted to solid-phase synthesis of 1⟶6- and 1⟶4-linked disaccharides in good to moderate yields.

Experimental

General

Mps were measured on a Danotherm melting point apparatus and are uncorrected. All solvents were distilled and/or stored over 3 Å or 4 Å molecular sieves as appropriate. ^{1}H-NMR spectra were recorded on either a Varian Mercury 300 operating at 300.06 MHz equipped with a 4-nuclei probe or a Varian Unity Inova 500 operating at 499.87 MHz equipped with a *z*- (single axis) PFG inverse detection C-H-P probe. ^{13}C-NMR spectra were recorded on a Varian Mercury 300 operating at 75.46 MHz. Chemical shift (δ)-values were in ppm and coupling constants (J) in Hz. All assignments were supported by 2D homonuclear chemical-shift correlation spectroscopy (gCOSY) and heteronuclear single quantum correlated spectroscopy (gHSQC) experiments. Thin-layer chromatography (TLC) was performed on Merck Silica Gel 60 F_{254} plates and spots were visualised by UV light at 254 nm and/or spraying with 10% aq. H_2SO_4 followed by heating. Molecular sieves (4 Å) for glycosylation reactions were crushed under argon followed by activation at 150 °C under high vacuum for 16 h. Vacuum liquid

chromatography (VLC) was carried out on Merck Silica Gel 60H. HPLC analyses were conducted with a Waters system [600 control unit, 996 photodiode array (PDA) detector, 717 Plus autosampler, Millenium32 control software] on a Waters Nova-Pak or XTerra C18 column (3.9 × 5.0 mm cartridge; 4 μm particle size) using a linear gradient of 0.1% aq. TFA (A) and 0.1% TFA in CH_3CN (B): 0 min: 0% B, 2 min: 0% B, 5 min: 50% B, 12 min: 95% B, 13 min: 95% B, 13.5 min: 0% B, 20 min: 0% B. Monitoring was from 200 to 400 nm, integrations were performed at 215 and 265 nm, and individual peaks were analyzed by their UV spectra. The purity of compounds was determined from integrations at 215 nm. MS analyses (electrospray, positive mode) were performed on a Micromass LCT mass spectrometer.

General method for model glycosylations and HPLC analysis (Tables 1 and 2)

The initial evaluation of solvents for the glycosylation was performed by treating glycosyl donor **1α,β** or **3α,β** (typically 0.02 mmol) with cyclohexanol and an activator in the solvent indicated (400–600 mm^3) on an Eppendorf Thermomixer 5436 (combined heater/shaker). After shaking and heating for the period indicated, a sample (10–40 mm^3) was diluted with CH_3CN (0.70–1.00 cm^3) and analyzed by analytical RP-HPLC. Reported yields were based on integrated areas at 215 nm.

General method for glycosylations and isolation (Table 3)

Glycosylation using 1α,β. Glycosyl donor (1.5 equiv.) and acceptor (**8**, **9**, **14**, or **19**; 0.1 mmol) were weighed off in a 5 cm^3 polypropylene test tube, mounted with a septum and needle, evacuated in high vacuum and filled with argon, repeated twice, and then dried under high vacuum for 1–2 h. 50–100 mg of 4 Å molecular sieves were added followed by addition of the solvent indicated (1 cm^3). The mixture was now agitated overnight to remove residual water present. The activator was added as a solution of $BF_3{\cdot}Et_2O$ in the solvent or as solid $LiClO_4$.

Glycosylations using 3β. Glycosyl donor (1.5–2.0 equiv.) and acceptor (**8**, **14**, or **19**; 0.1 mmol) were weighed off in 1.5 cm^3 Eppendorf centrifuge tubes together with $LiClO_4$, if required. Solvent indicated was added (1 cm^3), followed by addition of a solution of $BF_3{\cdot}Et_2O$ in the solvent, if required. The mixture was then agitated and heated on an Eppendorf Thermomixer 5436 (combined heater/shaker).

Purification. For reactions in CH_2Cl_2 or toluene the reaction mixture was diluted with CH_2Cl_2 (40 cm^3), washed successively with 1 M aq. HCl (2 × 20 cm^3) and 0.5 M aq. NaOH (2 × 20 cm^3), dried ($MgSO_4$) and concentrated. The residue was dissolved in CH_3CN, membrane filtered (0.45 μm) using CH_3CN, and products were isolated by preparative HPLC using a gradient of CH_3CN in water. For reactions in nitromethane, the reaction mixture was membrane filtered (0.45 μm) directly using CH_3CN without extraction prior to preparative HPLC. Appropriate fractions were concentrated *in vacuo* at 30 °C, traces of water were removed by co-evaporation once each with CH_3CN and CH_2Cl_2, and the product was dried under high vacuum to give the isolated yields stated.

General method for solid-phase glycosylations and determination of loadings (Table 4)

The resin (PS, 15–20 mg, ≈4–6 μmol acceptor), glycosyl donor (**1α,β** or **3β**), and 4 Å molecular sieves (≈50 mg) were weighed off in a 2 cm^3 disposable syringe fitted with a polypropylene filter and a Teflon valve. The syringes were mounted with septa and evacuated in high vacuum and then filled with argon. The procedure was repeated twice, followed by drying under high vacuum for 1–2 h. Upon inlet of argon, the valves were closed or if heating was required exchanged for a stopper, and solvent added (0.6–1.0 cm^3) followed by a pre-activation period (30–60 min) for removal of residual water by the molecular sieves. Lewis acid as indicated was either diluted in the solvent indicated and added with a Hamilton syringe ($BF_3{\cdot}Et_2O$) or added as solid ($LiClO_4$). Reactions that required heating were placed on an Eppendorf Thermomixer 5436 (combined heater–shaker) or on a well-equilibrated sand-bath. After agitation for 24 h (500 min^{-1}), the resin was washed successively with DMF (3 × 2 cm^3), CH_2Cl_2–MeOH (2 : 1; 3 × 2 cm^3), and CH_2Cl_2 (5 × 2 cm^3). Acetic anhydride (600 mm^3) and pyridine (300 mm^3) were added, and after shaking for 16 h the resin was washed as before. Acetylated product and remaining acceptor was released from the resin using TFA–water (19; 1, 0.5 cm^3) within 60 min. The cleavage mixture and washes with CH_2Cl_2 (5 × 2 cm^3) were collected and concentrated. The residue was dissolved in CH_3CN, diluted to 5.00 cm^3, membrane filtered (0.45 μm), and a sample injected into analytical HPLC. Cleaved amounts were quantified by integration from HPLC and comparison with standard curves (analytical standards of 2,3,4,6-tetra-*O*-benzyl-D-glucose and 1,2,3,4,6-penta-*O*-benzoyl-D-glucopyranose). Loadings of resin-bound acceptors were determined in the same fashion, but without the glycosylation step.

Synthesis of glycosyl donors 1α,β, 2α,β, and 3α,β

Glycosyl donors were synthesised as previously described.[7] Anomeric ratios in donors used: **1** (α/β 4.0 : 1), **2** (α/β 4.1 : 1), **3** (α/β 1 : 22.3).

Cyclohexyl 2,3,4,6-tetra-*O*-benzyl-α,β-D-glucopyranoside 4α,β

Observed NMR data were identical with literature values.[27]

Cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside 5α

Mp 91–92 °C; δ_H (300 MHz; $CDCl_3$) 8.04–7.82 (8 H, m), 7.60–7.22 (12 H, m), 6.19 (1 H, t, *J* 9.8, 3-H), 5.63 (1 H, t, *J* 9.7, 4-H), 5.50 (1 H, d, $J_{1,2}$ 3.9, 1-H), 5.26 (1 H, dd, $J_{2,3}$ 10.1 and $J_{1,2}$ 3.9, 2-H), 4.62–4.42 (3 H, m, 5-H and 6-H_2), 3.62 (1 H, m), 2.0–1.1 (10 H, m); δ_C (75 MHz; $CDCl_3$) 166.4, 166.0 (2 × C), 165.6, 134–127 (m), 94.9 (1α-C), 77.8, 72.4, 70.9, 70.0, 68.1, 63.5, 33.7, 31.8, 25.7, 24.2, 23.9; *m*/*z* 696.1 ($[M + NH_4]^+$. $C_{40}H_{42}NO_{10}$ requires *m*/*z*, 696.3).

Cyclohexyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside 5β

Mp 97.5–100 °C; δ_H (300 MHz, $CDCl_3$) 8.04 (8 H, m), 7.56 (12 H, m), 5.90 (1 H, t, *J* 9.5, 3-H), 5.64 (1 H, t, *J* 9.8, 4-H), 5.50 (1 H, dd, $J_{2,3}$ 9.8 and $J_{1,2}$ 7.9, 2-H), 4.94 (1 H, d, $J_{1,2}$ 7.8, 1-H), 4.62 (1 H, dd, J_{gem} 12.1 and $J_{5,6}$ 3.4, 6-H), 4.52 (1 H, dd, J_{gem} 12.2 and $J_{5,6}$ 6.0, 6-H′), 4.19–4.10 (1 H, m, 5-H), 3.72–3.62 (1 H, m), 2.0–1.1 (10H, m); δ_C (75 MHz; $CDCl_3$) 166.3, 166.0, 165.4, 165.2, 134–127 (m), 100.1 (1β-C), 78.7, 73.3, 72.4, 72.3, 70.4, 63.7, 33.5, 31.9, 25.6, 23.9, 23.8; *m*/*z* 696.1 ($[M + NH_4]^+$. $C_{40}H_{42}NO_{10}$ requires *m*/*z*, 696.3).

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α,β-D-glucopyranosyl)-α-D-glucopyranoside 10α,β

Isolated yield after preparative HPLC: 82% (α/β ≈ 1 : 1). Observed NMR data were identical with literature values.[27]

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α,β-D-glucopyranosyl)-α-D-glucopyranoside 11α,β

Isolated yield after preparative HPLC: 34% (α/β 1 : 3.3). Observed NMR data were identical with literature values.[27]

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-α-D-glucopyranoside 12

Isolated yield after preparative HPLC: 46% (pure β). Observed NMR data were identical with literature values.[27]

Methyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside[28] 14

Trityl ether **13** (658 mg, 1.0 mmol) was dissolved in THF (5 cm^3), 3 M aq. HCl (1.0 cm^3) was added, and the reaction mixture was heated for 17 h at 50 °C by means of an oil-bath. The solution was then diluted with ethyl acetate (40 cm^3), washed once with brine (20 cm^3), dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was then subjected to VLC on silica gel (CH_2Cl_2–acetone 4 : 1) and fractions containing a compound with R_f 0.12 (CH_2Cl_2–acetone 4 : 1) were concentrated *in vacuo*. The resulting gel was lyophilised from pentane to yield **14** (292 mg, 70%) as a colorless solid, mp 181–182 °C (lit.,[28] 186–188 °C); purity > 99% (HPLC); observed NMR data were identical with literature values.[28]

Methyl 2-amino-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside 15

Trityl ether **13** (658 mg, 1.0 mmol) was dissolved in THF (5 cm^3), 3 M aq. HCl (2.5 cm^3) was added, and the reaction mixture was heated at reflux for 3 d by means of an oil-bath. The solution was then diluted with ethyl acetate (40 cm^3) and extracted with 1 M aq. HCl (2 × 20 cm^3). The combined aq. phases were treated with conc. NaOH (28%) until pH > 11 and extracted with ethyl acetate (2 × 20 cm^3). The combined organic phases were washed with brine (10 cm^3), dried (Na_2SO_4), and concentrated *in vacuo*. The crude syrup was then subjected to VLC on silica gel (CH_2Cl_2–acetone–MeOH 75 : 20 : 5) and fractions containing a compound with R_f 0.25 (CH_2Cl_2–acetone–MeOH 72.5 : 20 : 7.5) were concentrated *in vacuo* to yield amine **15** as a colorless syrup that solidified on storage and was lyophilised from pentane (226 mg, 61%), mp 68–70 °C; purity > 97% (HPLC); δ_H (500 MHz; $CDCl_3$) 7.36–7.25 (10 H, m), 4.98 (1 H, d, J_{gem} 11.3), 4.87 (1 H, d, J_{gem} 11.0), 4.75–4.67 (3 H, m, 2 × PhCH_2 + 1-H), 3.83 (1H, dd, J_{gem} 11.5 and $J_{5,6}$ 2.1, 6-H), 3.75 (1 H, dd, J_{gem} 11.9 and $J_{5,6}$ 3.4, 6-H′), 3.71–3.67 (1 H, m, 5-H), 3.59 (1 H, t, *J* 9.0, 3-H), 3.54 (1 H, t, *J* 9.0, 4-H), 3.36 (3 H, s, OMe), 2.77 (1 H, dd, $J_{2,3}$ 9.0 and $J_{1,2}$ 3.4, 2-H); δ_C (75 MHz; $CDCl_3$) 138.8, 138.3, 129–128 (m), 100.9 (1α-C), 84.1, 78.9, 75.9, 75.0, 71.7, 62.1, 56.4, 55.4; *m*/*z* 374.19 ($[M + H]^+$. $C_{21}H_{28}NO_5$ requires *m*/*z*, 374.20).

Methyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α,β-D-glucopyranosyl)-α-D-glucopyranoside 16α,β

Isolated yield after preparative HPLC: Activation by $BF_3{\cdot}Et_2O$ (77%, α/β 1.4 : 1) and $LiClO_4$ (93%, α/β 1.6 : 1). The two anomers proved inseparable by preparative HPLC; purity > 99% (HPLC); selected NMR-data: δ_H (300 MHz; $CDCl_3$) 7.42–7.12 (30 H, m, Bn), 5.27 (1 H, 2 d, NH), 3.32 (s, α-OMe) and 3.29 (s, β-OMe), 1.87 (3 H, s, NAc); δ_C (75 MHz; $CDCl_3$) **16α**: 104.1 (1′β-C), 98.8 (1α-C); **16β**: 97.5 (1′α-C), 98.6 (1α-C); *m*/*z* (anomeric mixture) 960.43 ($[M + Na]^+$. $C_{57}H_{63}NNaO_{11}$ requires *m*/*z*, 960.43).

Methyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-α-D-glucopyranoside 17β

Isolated yield after preparative HPLC: 91% (colorless solid); mp 132–133 °C; purity > 99% (HPLC); observed ^{1}H-NMR data were identical with literature values:[14] δ_C (75 MHz; $CDCl_3$) 169.7, 166.3, 166.0, 165.4, 165.1, 138.5, 138.1, 133.6, 133.4, 133.33, 133.29, 130.0–127.9 (m), 101.7, 98.5, 80.5, 78.6, 75.0, 74.7, 73.1, 72.5, 72.1, 70.4, 70.1, 68.9, 63.5, 54.9, 52.5, 23.7; *m*/*z* 1016.36 ($[M + Na]^+$. $C_{57}H_{55}NNaO_{15}$ requires *m*/*z*, 1016.35).

Methyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside 19

Benzylidene **18** was ring-opened by the method of Debenham and Toone[20] using Et_3SiH and $BF_3{\cdot}Et_2O$. Isolated yield of **19** after preparative HPLC: 74% (colorless solid), mp 133–135 °C [lit.,[29] 144–145 (from acetone–Et_2O–hexane)]; purity > 99% (HPLC); δ_H (300 MHz; $CDCl_3$) 7.40–7.25 (10 H, m), 5.42 (1 H, d, *J* 9.0, NH), 4.73 (2 H, m, PhCH_2), 4.68 (1 H, d, *J* 3.7, 1α-H), 4.59 (2 H, m, PhCH_2), 4.22 (1 H, ddd, *J* 10.3, 9.0 and 3.7, 2-H), 3.80–3.68 (4 H, m, 4-H, 5-H and 6-H_2), 3.56 (1 H, dd, *J* 10.3 and 7.8, 3-H), 3.34 (3 H, s, OMe), 2.82 (1 H, d, *J* 2.1, OH), 1.90 (3 H, s, NAc); δ_C (75 MHz; $CDCl_3$) 170.0, 138.7, 138.1, 128.7–127.8 (m), 98.9, 80.1, 74.0, 73.9, 72.4, 70.6, 70.9, 55.3, 52.1, 23.7.

Methyl 2-amino-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside 20

Adapting a procedure by Charette and Chua,[23] benzylidene **18** (827 mg, 2.0 mmol) in a dry flask was dissolved in dry CH_2Cl_2 (30 cm^3) under argon. Dry pyridine (0.50 cm^3, 6.0 mmol) was added and the reaction mixture was cooled to −40 °C by means of a bath of solid CO_2 in CH_3CN. Tf_2O (0.50 cm^3, 3.0 mmol) was added dropwise with a syringe under stirring and the mixture was allowed to warm to 0 °C over a period of 2.5 h. After another 4 h at 0 °C, dry EtOH (5 cm^3) was added and the mixture warmed to rt overnight. Additional CH_2Cl_2 (20 cm^3) was added and the organic phase was washed successively with 0.5 M aq. HCl (2 × 15 cm^3) and saturated aq. $NaHCO_3$ (2 × 15 cm^3), dried (Na_2SO_4), and concentrated to a slightly yellow solid (0.79 g, from Et_2O–pentane). The residue was purified by VLC (CH_2Cl_2–MeOH 99 : 1) and fractions showing R_f 0.29 (CH_2Cl_2–MeOH 95 : 5) were concentrated to give **20** (621 mg, 84%) as a colorless solid, mp 104–110 °C; δ_H (300 MHz; $CDCl_3$) 7.55–7.10 (10 H, m), 5.59 (1 H, s), 5.05 (1 H, d, *J* 11.5), 4.75 (1 H, d, *J* 3.7, 1α-H), 4.68 (1 H, d, *J* 11.4), 4.29 (1 H, dd, *J* 9.5 and 4.1, 6-H), 3.90–3.73 (2 H, m, 5-H and 6-H′), 3.70–3.58 (2 H, m, 3- and 4-H), 3.40 (3 H, s, OMe), 2.86 (1 H, dd, *J* 9.2 and 3.7, 2-H), 1.57 (2 H, br s, NH_2); δ_C (75 MHz; $CDCl_3$) 138.8, 137.8, 129.1–127.9 (m), 126.3, 126.2, 101.5, 101.4, 83.5, 80.2, 75.2, 69.5, 63.1, 56.2, 55.5; *m*/*z* 372.17 ($[M + H]^+$. $C_{21}H_{26}NO_5$ requires *m*/*z*, 372.18).

Methyl 2-amino-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside 21

Benzylidene **20** was ring-opened by the method of Debenham and Toone[20] using triethylsilane and $BF_3{\cdot}Et_2O$ (3 equiv.) for 16 h. Isolated yield of **21** after VLC (CH_2Cl_2–MeOH 99 : 1): 55% (colorless syrup); purity > 98% (HPLC); δ_H (300 MHz; $CDCl_3$) 7.40–7.25 (10 H, m), 4.92 (1 H, d, *J* 11.9, PhCH_2), 4.77 (1 H, d, *J* 11.9, PhCH_2), 4.70 (1 H, d, *J* 3.4, 1α-H), 4.63 (1 H, d, *J* 12.0, PhCH_2), 4.55 (1 H, d, *J* 12.0, PhCH_2), 3.78–3.61 (4 H, m, 4-H, 5-H and 6′-H_2), 3.42 (1 H, dd, *J* 9.9 and 8.4, 3-H), 3.38 (3 H, s, OMe), 2.77 (1 H, dd, *J* 9.9 and 3.4, 2-H), ≈1.5 (≈2 H, br s, NH_2); δ_C (75 MHz; $CDCl_3$) 139.0, 138.1, 128.8–127.9 (m), 101.0, 84.1, 75.5, 74.0, 73.3, 70.8, 70.3, 55.6, 55.4; *m*/*z* 374.19 ($[M + H]^+$. $C_{21}H_{28}NO_5$ requires *m*/*z*, 374.20).

Methyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α,β-D-glucopyranosyl)-α-D-glucopyranoside 22α,β

Isolated yield after preparative HPLC: 82%, α/β 1.9 : 1. The two anomers proved inseparable by preparative HPLC; purity > 99% (HPLC); selected NMR-data: δ_H (300 MHz; $CDCl_3$) 7.20–7.00 (30 H, m, Ph), 5.45 (0.65 H, d, *J* 3.5, 1α-H), 5.35 (0.65 H, d, *J* 9.1, α-NH), 5.14 (0.35 H, d, *J* 8.9, β-NH), 3.31 (1.95 H, s, α-OMe), 3.28 (1.05 H, s, β-OMe), 1.78 (1.95 H, α-Ac), 1.77 (1.05 H, s, β-Ac); δ_C (75 MHz; $CDCl_3$) 102.9 (**22β**, 1′β-C), 98.6 (**22β**, 1α-C), 98.4 (**22α**, 1′α-C), 97.1 (**22α**, 1α-C); *m*/*z* (anomeric mixture) 960.43 ($[M + Na]^+$. $C_{57}H_{63}NNaO_{11}$ requires *m*/*z*, 960.43).

Methyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-α-D-glucopyranoside 23β

Isolated yield after preparative HPLC: 35%, pure β; purity

> 98% (HPLC); δ_H (500 MHz; $CDCl_3$) 8.02–7.78 (8 H, m, Ph), 7.58–7.20 (22 H, m, Ph), 5.70 (1 H, dd, *J* 9.8, 3′-H), 5.58 (1 H, dd, *J* 9.8, 4′-H), 5.53 (1 H, dd, *J* 9.8 and 7.7, 2′-H), 5.10 (1 H, d, *J* 8.5, NH), 4.96 (1 H, d, *J* 12.4, PhCH_2), 4.85 (1 H, d, *J* 7.7, 1′α-H), 4.78 (1 H, d, *J* 11.9, PhCH_2), 4.69 (1 H, d, *J* 3.8, 1α-H), 4.64 (1 H, d, *J* 11.9, PhCH_2), 4.54 (1 H, dd, *J* 11.9 and 3.0, 6′-H[a]), 4.41 (1 H, d, *J* 12.4, PhCH_2), 4.27 (1 H, dd, *J* 11.9 and 5.6, 6′-H[b]), 4.13 (1 H, ddd, *J* 10.7, 8.5, and 3.8, 2-H), 4.10 (1 H, t, *J* 9.0, 4-H), 3.81 (1 H, ddd, *J* 9.8, 5.6, and 3.0, 5′-H), 3.73 (1 H, dd, *J* 11.1 and 3.0, 6-H[a]), 3.58 (1 H, dd, *J* 10.7 and 9.0, 3-H), 3.50–3.43 (2 H, m, 5-H and 6-H[b]), 3.21 (3 H, s, OMe), 1.77 (3 H, s, OAc); δ_C (75 MHz; $CDCl_3$) 169.9, 166.3, 165.9, 165.3, 165.1, 138.2, 138.3, 133.6 (2 × C), 133.4, 133.3, 130–128.3 (m), 127.7, 100.8, 98.6, 77.9, 77.5, 74.5, 73.8, 73.4, 72.6, 72.2, 70.4, 70.0, 67.7, 63.1, 55.4, 52.6, 23.5; *m*/*z* 1016.36 ([M + Na]$^+$. $C_{57}H_{55}NNaO_{15}$ requires *m*/*z*, 1016.35).

Methyl 2-acetamido-6-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside 27

From a determination of loading of acceptor **25**, the cleavage mixture was concentrated, and dissolved in $CDCl_3$: purity > 99% (HPLC); δ_H (500 MHz; $CDCl_3$) 7.37–7.28 (10 H, m, PhCH_2), 5.30 (1 H, d, 9.4, NH), 4.87 (1 H, d, *J* 11.1, PhCH_2), 4.85 (1 H, d, *J* 11.9, PhCH_2), 4.66 (1 H, d, *J* 11.5, PhCH_2), 4.64 (1 H, d, *J* 3.4, 1α-H), 4.60 (1 H, d, *J* 11.1, PhCH_2), 4.34 (1 H, dd, *J* 11.9 and 2.1, 6-H), 4.26 [1 H, dt, *J* 9.4 (2 ×) and 3.4, 2-H], 4.24 (1 H, dd, *J* 11.9 and 4.3, 6-H′), 3.79 (1 H, ddd, *J* 9.8, 4.3, and 2.1, 5-H), 3.70 (1 H, dd, *J* 10.2 and 9.0, 3-H), 3.62 (1 H, dd, *J* 9.8 and 9.0, 4-H), 3.34 (3 H, s, OMe), 2.06 (3 H, s, OAc), 1.86 (3 H, s, NAc); δ_C (75 MHz; $CDCl_3$, selected data) 98.9, 80.6, 78.3, 75.3, 75.2, 69.4, 63.0, 55.3, 52.7, 23.7, 21.1; *m*/*z* 480.19 ([M + Na]$^+$. $C_{25}H_{31}NNaO_7$ requires *m*/*z*, 480.19).

Methyl 2-acetamido-4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside 28

From a determination of loading of acceptor **26**, the cleavage mixture was concentrated, and dissolved in $CDCl_3$: purity > 97% (HPLC); δ_H (300 MHz; $CDCl_3$) 7.37–7.21 (10 H, m, Ph), 5.33 (1 H, *J* 9.4, NH), 5.14 (1 H, *J* 10.1 and 9.3, 4-H), 4.74 (1 H, *J* 3.6, 1α-H), 4.62 (1 H, *J* 11.5, PhCH_2), 4.57–4.49 (3 H, m, PhCH_2), 4.34 (1 H, ddd, *J* 10.5, 9.0, and 3.4, 2-H), 3.84 (1 H, dt, *J* 10.1 and 4.4, 5-H), 3.73 (1 H, dd, *J* 10.6 and 9.3, 3-H), 3.54 (2 H, d, *J* 4.4, 6-H_2), 3.38 (3 H, s, OMe), 1.93 (3 H, s, OAc), 1.88 (3 H, s, NAc); δ_C (75 MHz; $CDCl_3$, selected data) 98.6, 77.8, 77.4, 73.8, 72.6, 71.0, 69.5, 55.4, 51.9; *m*/*z* 480.19 ([M + Na]$^+$. $C_{25}H_{31}NNaO_7$ requires *m*/*z*, 480.19).

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References

1 Preliminary accounts of this work have been reported: L. Petersen and K. J. Jensen, presented at the 20th International Carbohydrate Symposium (ICS), Hamburg, Germany, 2000, August 27–September 1, Poster B-191.
2 The following abbrevations are used: Trimethylsilyl trifluoromethanesulfonate (TMSOTf), 5-(2-formyl-3,5-dimethoxyphenoxy)-valeric acid (*o*-PALdehyde), *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HBTU), 1-hydroxybenzotriazole (HOBt), glycosyl donors derived from *di*nitro*sal*icylic acid or its regioisomer (DISAL), 1-methylpyrrolidin-2-one (NMP), polystyrene (PS), poly(ethylene glycol) polyacrylamide (PEGA).
3 (*a*) A. Varki, *Glycobiology*, 1993, **3**, 97; (*b*) *Molecular Glycobiology*, ed. M. Fukuda and O. Hindsgaul, IRL Press, Oxford, 1994; (*c*) K. J. Yarema and C. R. Bertozzi, *Curr. Opin. Chem. Biol.*, 1998, **2**, 49.
4 (*a*) P. Lerouge, P. Roche, C. Faucher, F. Maillet, G. Truchet, J. C. Promé and J. Dénarié, *Nature*, 1990, **344**, 781; (*b*) I. M. Lopez-Lara, J. D. J. van den Berg, J. E. Thomas-Oartes, J. Glushka, B. J. J. Lugtenberg and H. P. Spaink, *Mol. Microbiol.*, 1995, **15**, 627; (*c*) J. Dénarié, F. Debellé and J.-C. Promé, *Annu. Rev. Biochem.*, 1996, **65**, 503.
5 (*a*) U. Schmid and H. Waldmann, *Chem. Eur. J.*, 1998, **4**, 494; (*b*) H. Schene and H. Waldmann, *Chem. Commun.*, 1998, 2759; (*c*) H. Schene and H. Waldmann, *Eur. J. Org. Chem.*, 1998, 1227; (*d*) H. Schene and H. Waldmann, *Synthesis*, 1999, 1411.
6 A. Lubineau and B. Drouillat, *J. Carbohydr. Chem.*, 1997, **16**, 1179.
7 (*a*) L. Petersen and K. J. Jensen, *J. Org. Chem.*, 2001, **66**, in press; (*b*) L. Petersen and K. J. Jensen, presented at the 20th International Carbohydrate Symposium (ICS), Hamburg, Germany, 2000, August 27–September 1, Poster B-193.
8 Aryl glycosides of salicylic acid are significantly more labile under acidic hydrolysis than are the corresponding phenyl glycosides. This has been ascribed to acid catalysis by the free benzoic acid moiety, see (*a*) B. Capon, *Tetrahedron Lett.*, 1963, 911; (*b*) B. Capon, M. C. Smith, E. Anderson, R. H. Dahm and G. H. Sankey, *J. Chem. Soc. B*, 1969, 1038.
9 J. B. Laursen, L. Petersen and K. J. Jensen, *Org. Lett.*, 2001, **3**, 687.
10 G. Barany and M. Kempe, in *A Practical Guide to Combinatorial Chemistry*, ed. A. W. Czarnik and S. H. DeWitt, American Chemical Society, Washington, 1997, p. 51.
11 For reviews see (*a*) P. H. H. Hermkens, H. C. J. Ottenheijm and D. Rees, *Tetrahedron*, 1996, **52**, 4527; (*b*) P. H. H. Hermkens, H. C. J. Ottenheijm and D. Rees, *Tetrahedron*, 1997, **53**, 5643; (*c*) S. Booth, P. H. H. Hermkens, H. C. J. Ottenheijm and D. Rees, *Tetrahedron*, 1998, **54**, 15385.
12 (*a*) N. K. Terret, *Combinatorial Chemistry*, Oxford University Press, Oxford, 1998; (*b*) B. A. Bunin, *The Combinatorial Index*, Academic Press, San Diego, 1998.
13 Some examples are: (*a*) S. J. Danishefsky, K. F. McClure, J. T. Randolph and R. B. Ruggeri, *Science*, 1993, **260**, 1307; (*b*) L. Yan, C. M. Taylor, R. Goodnow and D. Kahne, *J. Am. Chem. Soc.*, 1994, **116**, 6953; (*c*) S. P. Douglas, D. M. Whitfield and J. J. Krepinsky, *J. Am. Chem. Soc.*, 1995, **117**, 2116; (*d*) J. A. Hunt and W. R. Roush, *J. Am. Chem. Soc.*, 1996, **118**, 9998; (*e*) J. Rademann and R. R. Schmidt, *J. Org. Chem.*, 1997, **62**, 3650; (*f*) R. Rodebaugh, S. Joshi, B. Fraser-Reid and H. M. Geysen, *J. Org. Chem.*, 1997, **62**, 5560; (*g*) S. Mehta and D. Whitfield, *Tetrahedron Lett.*, 1998, **39**, 5907; (*h*) P. H. Seeberger and S. J. Danishefsky, *Acc. Chem. Res.*, 1998, **31**, 685; (*i*) R. B. Andrade, O. J. Plante, L. G. Melean and P. H. Seeberger, *Org. Lett.*, 1999, **1**, 1811; (*j*) F. Roussel, L. Knerr, M. Grathwohl and R. R. Schmidt, *Tetrahedron Lett.*, 2000, **41**, 3043; (*k*) O. J. Plante, E. R. Palmacci and P. H. Seeberger, *Science*, 2001, **291**, 1523.
14 J. F. Tolborg and K. J. Jensen, *Chem. Commun.*, 2000, 147.
15 For a recent review, see P. H. Seeberger and W.-C. Haase, *Chem. Rev.*, 2000, **100**, 4349.
16 S. K. Chatterjee and P. Nuhn, *Chem. Commun.*, 1998, 1729.
17 This has been used to generate HF *in situ* for the removal of silyl protecting groups: S. Mabic and J.-P. Lepoittevin, *Synlett*, 1994, 851.
18 R. Caputo, H. Kunz, D. Mastroianni, G. Palumbo, S. Pedatella and F. Solla, *Eur. J. Org. Chem.*, 1999, 3147.
19 K. Umemura, H. Matsuyama, M. Kobayashi and N. Kamigata, *Bull. Chem. Soc. Jpn.*, 1989, **62**, 3026.
20 S. D. Debenham and E. J. Toone, *Tetrahedron: Asymmetry*, 2000, **11**, 385.
21 R. W. Jeanloz, *J. Am. Chem. Soc.*, 1952, **74**, 4597.
22 A. Roy, A. K. Ray, A. Mukherjee and N. Roy, *Indian J. Chem., Sect. B*, 1987, **26**, 1165.
23 A. B. Charette and P. Chua, *Synlett*, 1998, 163.
24 (*a*) K. J. Jensen, J. Alsina, M. F. Songster, J. Vágner, F. Albericio and G. Barany, *J. Am. Chem. Soc.*, 1998, **120**, 5441; (*b*) J. Alsina, K. J. Jensen, F. Albericio and G. Barany, *Chem. Eur. J.*, 1999, **5**, 2787; (*c*) J. Alsina, K. J. Jensen, M. F. Songster, J. Vagner, F. Albericio and G. Barany, in *Solid Phase Organic Syntheses*, ed. A. W. Czarnik, Wiley, New York, 2000, vol. 1, p. 121.
25 Y. Sasaki and D. H. Coy, *Peptides (Pergamon)*, 1987, **8**, 119.
26 J. F. Tolborg, L. Petersen, K. J. Jensen, C. Mayer, D. L. Jakeman, R. A. J. Warren and S. G. Withers, *in preparation*.
27 W.-S. Kim, S. Hosono, H. Sasai and M. Shibasaki, *Heterocycles*, 1996, **42**, 795.
28 M. L. Falcone-Hindley and J. T. Davis, *J. Org. Chem.*, 1998, **63**, 5555.
29 S. S. Rana, J. J. Barlow and K. L. Matta, *Carbohydr. Res.*, 1983, **113**, 257.